A STUDY OF THE BETA HEMOLYSIN OF STAPHYLOCOCCI

BY

ROBERT ALLEN ALTENBERN

B.S., University of New Hampshire, 1943
M.S., University of Illinois, 1947

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BACTERIOLOGY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1949

URBANA, ILLINOIS

1949

A STUDY OF THE BETA HEMOLYSIN OF STAPHYLOCOCCI

BY

ROBERT ALLEN ALTENBERN

B.S., University of New Hampshire, 1943
M.S., University of Illinois, 1947

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BACTERIOLOGY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1949

URBANA, ILLINOIS

1949

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Professor F. M. Clark for constant advice and encouragement throughout the course of this investigation. He is very grateful to Professor H. H. Thornberry, Department of Horticulture, for generous cooperation during precipitation studies at low temperature and to Mr. Ray Hamilton, College of Veterinary Medicine, for supplying fresh sheep's blood at regular intervals. In less tangible ways, the author owes a great debt to other staff members and fellow graduate students for their many helpful suggestions and criticisms.

R. UNIV. BIBLIOTHEEK LEIDEN

A STUDY OF THE BETA HEMOLYSIN OF STAPHYLOCOCCI

Introduction

The unique action of beta hemolysin was first noted by Bigger, Boland and O'Meara who termed this action, "hot-cold" hemolysis. A precise characterization of the activity of beta hemolysin was made by Glenny and Stevens and by Bryce and Rountree. This hemolysin is active against erythrocytes only of bovine origin and one hour incubation at 37°C. followed by six to eight hour refrigeration (4-6°C.) is necessary to demonstrate its hemolytic activity.

The chemical properties of this substance have been inadequately investigated. It can be precipitated by ammonium sulfate, trichloracetic acid or acetone, it is non dialyzable and is destroyed by trypsin. Application of precise chemical methods to purification of other bacterial toxins has been fruitful, especially with botulinum toxin and the toxin produced by the diphtheria bacillus. Recently methanol precipitation of staphylococcal alpha toxin under rigidly controlled conditions of ionic strength, pH and temperature has been successful.

A study of kinetics and mechanism of other bacterial hemolysins, particularly the hemolysin produced by *Clostridium septicum,* has yielded much information about the true nature of hemolysis by bacterial toxins.

There has been apparently little study of the beta hemolysin of staphylococci with regard to its characteristics as a molecule or to its action on bovine erythrocytes.

Experimental

A strain of *Staphylococcus aureus,* F-2-35, which is known to produce large amounts of beta hemolysin, was employed throughout this study. Toxin was produced in tryptose

phosphate broth (Difco) plus 0.2% starch which, after inoculation, was incubated under 20% carbon dioxide and 80% air for 48 hours at 37°C. At the end of the incubation period, cells were removed from the broth by centrifugation and the clear supernatant fluid was stored for use. This preparation was labeled crude toxin. Titrations for beta hemolytic activity were made against a 1% suspension of fresh, washed sheep erythrocytes.

Ammonium sulfate precipitation studies on crude toxin showed that beta hemolysin may be precipitated quantitatively by 68% saturation with ammonium sulfate or 2.80 moles per liter. The ammonium sulfate was introduced by dialysis for 48 hours through Visking casing. Although beta hemolysin may be precipitated by this method, many colored constituents of the medium are simultaneously precipitated and resolution with ammonium sulfate alone is unsatisfactory.

Addition of mineral acid to crude toxin produces a fine, white precipitate which, at a favorable pH, contains most of the beta hemolysin. Adjustment of equal volumes of crude toxin to varying pH values with 0.2 N hydrochloric acid showed that pH 3.5 is optimal for precipitation of this hemolysin. There are, however, considerable losses of activity in this procedure but none of the colored constituents of the medium is precipitated by this method. Ammonium sulfate fractionation of acid precipitated beta hemolysin resulted in greater yields at low concentrations of ammonium sulfate. A concentration of 1.68 moles per liter or 40% saturation with this salt precipitated almost all of the beta hemolysin. Recoveries were still of a low order.

Fractionation with methanol under controlled conditions of temperature, pH and ionic strength yielded promising results. Beta hemolysin may be precipitated with 50-100% recovery by 25-30 volume percent methanol, ionic strength of 0.11-0.13 and pH of 4.0-4.7 at a temperature of −5°C. Precipitates are white or slightly gray and contain no colored medium constituents.

Inhibition studies were entirely negative. Mercuric ions, sodium iodoacetate, hydrogen peroxide, sodium cyanide and sodium azide were all ineffective as inhibitors of beta hemolysin. Since the first three of these will inhibit enzymes which require free sulfhydryl groups, beta hemolysin appears not to be a sulfhydryl enzyme.

After primary incubation at 37°C. for one hour, hemolysin-exposed sheep erythrocytes exhibited slight color changes and increased transparency macroscopically. Upon microscopic observation hemolysin-exposed erythrocytes were uniformly swollen, light in color and possessed a perfectly circular periphery. Control cells were darker in color and had a notched edge and wrinkled surface, a characteristic appearance of slightly crenated erythrocytes. By increasing osmotic pressure with concentrated sugar solutions, sucrose, fructose or glucose, hemolysis could be delayed or completely prevented. A final concentration of 0.24 molar sucrose delayed hemolysis whereas 0.98 molar sucrose would prevent hemolysis completely.

The specific action of beta hemolysin is complete after preliminary incubation at 37°C. If sheep erythrocytes were removed from suspension by centrifuging after this preliminary incubation with hemolysin, and resuspended in an equal volume of 0.9% saline, typical hemolysis occurred upon refrigeration.

The interaction between beta hemolysin molecules and sheep erythrocytes appears to be a simple adsorption phenomenon. No maximum adsorption of beta hemolysin could be demonstrated. In general this reaction resembles a Freundlich adsorption isotherm. The equation for this process is $Y = 1.03\ X^{(0.71)}$ where Y is the amount of hemolysin adsorbed per 1.0 ml. of 1% sheep cell suspension and X is the amount of hemolysin remaining free at equilibrium.

Summary

Staphylococcal beta toxin may be precipitated by ammo-

nium sulfate, mineral acid or in the cold with methanol. It is iso-electric at pH 3.5. A concentration of methanol of 25-30 volume percent, ionic strength of 0.11-0.13 and pH of 4.0-4.7 is favorable for precipitation of beta hemolysin at — 5°C.

Mercuric ions, sodium iodoacetate, hydrogen peroxide, sodium cyanide and sodium azide were all ineffective as inhibitors of beta hemolysin.

The specific action of beta hemolysin is complete after one hour incubation at 37°C. Sucrose, fructose and glucose in suitable concentrations can delay or prevent hemolysis. The reaction between beta hemolysin and sheep erythrocytes may be expressed as a Freundlich adsorption isotherm.

REFERENCES

1. Bigger, J. W., Boland, C. R. and O'Meara, R. A. Q. J., Path. Bact. 30: 271 (1927)

2. Glenny, A. T. and Stevens, M. F., J. Path Bact. 40: 201 (1935)

3. Bryce, L. M. and Rountree, P. M., J. Path. Bact. 43: 173 (1936)

VITA

The author was born June 12, 1923 in Junction City, Kansas. He was graduated from Woodsville (N.H.) High School in 1940. He attended the University of New Hampshire and received a Bachelor of Science degree in Bacteriology in 1943. From October, 1943 to May, 1944 he was a graduate assistant in Bacteriology at the University of Illinois. In July, 1944 he was called to duty in the U. S. Naval Reserve and received an honorable discharge in August, 1946. He returned to the University of Illinois as a graduate assistant in Bacteriology and received a Master of Science degree in Bacteriology in June, 1947. Until June, 1948 he remained a graduate assistant in this department. A research fellowship was awarded him for the period October, 1948 to February, 1949. The author is a member of Phi Sigma, Sigma Xi, Society of American Bacteriologists and American Association for the Advancement of Science.

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41549807_0118